Date of Approval: December 8, 2011

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-333

SENTINEL SPECTRUM

milbemycin oxime/lufenuron/praziquantel

Dogs

SENTINEL SPECTRUM is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*; for the prevention and control of flea populations (*Ctenocephalides felis)*; and for the treatment and control of adult roundworm *(Toxocara canis*, *Toxascaris leonina)*, adult hookworm *(Ancylostoma caninum)*, adult whipworm *(Trichuris vulpis),* and adult tapeworm *(Taenia pisiformis, Echinococcus multilocularis* and *Echinococcus granulosus)* infections in dogs and puppies two pounds of body weight or greater and six weeks of age and older.

Sponsored by:

Novartis Animal Health US, Inc.

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I. GENERAL INFORMATION:

A. File Number: NADA 141-333

B. Sponsor: Novartis Animal Health US, Inc.
3200 Northline Ave., suite 300
Greensboro, NC 27408

Drug Labeler Code: 058198

C. Proprietary Name: SENTINEL SPECTRUM

D. Established Name: Milbemycin oxime/lufenuron/praziquantel

E. Pharmacological Category: Antiparasitic

F. Dosage Form: Chewable Tablet

G. Amount of Active Ingredients: Each chewable tablet contains:

2.3 mg milbemycin oxime/46 mg lufenuron/22.8 mg praziquantel
5.75 mg milbemycin oxime/115 mg lufenuron/57 mg praziquantel
11.5 mg milbemycin oxime/230 mg lufenuron/114 mg praziquantel
23 mg milbemycin oxime/460 mg lufenuron/228 mg praziquantel

H. How Supplied: SENTINEL SPECTRUM is available in four strengths according to the weight of the dog, in packages of six or twelve chewable tablets each, as listed below:

2.3 mg milbemycin oxime/46 mg lufenuron/22.8 mg praziquantel
5.75 mg milbemycin oxime/115 mg lufenuron/57 mg praziquantel
11.5 mg milbemycin oxime/230 mg lufenuron/114 mg praziquantel
23 mg milbemycin oxime/460 mg lufenuron/228 mg praziquantel

I. How Dispensed: Rx

J. Dosage(s):

SENTINEL SPECTRUM is given orally, once a month, at the minimum dosage of 0.23 mg/pound body weight (0.5 mg/kg) of milbemycin oxime, 4.55 mg/pound (10 mg/kg) of lufenuron, and 2.28 mg/pound (5 mg/kg) of praziquantel. For heartworm prevention, give once monthly beginning within 1 month of the dog's first seasonal exposure to mosquitoes and continuing until at least 6 months after the dog's last seasonal exposure.

Body Weight	Milbemycin Oxime Per Chewable	Lufenuron Per Chewable	Praziquantel Per Chewable	Number of Chewables
2 to 8 lbs.	2.3 mg	46 mg	22.8 mg	One
8.1 to 25 lbs.	5.75 mg	115 mg	57 mg	One
25.1 to 50 lbs.	11.5 mg	230 mg	114 mg	One
50.1 to 100 lbs.	23 mg	460 mg	228 mg	One
Over 100 lbs.	Administer the appropriate combination of chewables			

K. Route of Administration

Oral

L. Species:

Dogs

M. Indications:

SENTINEL SPECTRUM is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis*; for the prevention and control of flea populations (*Ctenocephalides felis)*; and for the treatment and control of adult roundworm *(Toxocara canis, Toxascaris leonina)*, adult hookworm *(Ancylostoma caninum)*, adult whipworm *(Trichuris vulpis),* and adult tapeworm *(Taenia pisiformis, Echinococcus multilocularis* and *Echinococcus granulosus)* infections in dogs and puppies two pounds of body weight or greater and six weeks of age and older.

II. EFFECTIVENESS:

A. Dosage Characterization:

The dose of milbemycin oxime necessary for the prevention of heartworm (*Dirofilaria immitis*) disease and adult hookworm (*Ancylostoma caninum*), roundworm (*Toxocara canis*, *Toxascaris leonina*), and whipworm (*Trichuris vulpis*) infections in dogs has been determined to be 0.5 mg/kg body weight under NADA 140-915, INTERCEPTOR (milbemycin oxime) Flavor Tabs (Novartis Animal Health US, Inc.) and NADA 141-084, SENTINEL (milbemycin oxime/lufenuron) Flavor Tabs (Novartis Animal Health US, Inc.).

The dose of lufenuron necessary for the prevention and control of flea populations (*Ctenocephalides felis*) in dogs has been determined to be 10 mg/kg body weight under NADA 141-035, PROGRAM (lufenuron) Flavor Tabs (Novartis Animal Health US, Inc.) and under NADA 141-084, SENTINEL (milbemycin oxime/lufenuron) Flavor Tabs (Novartis Animal Health US, Inc.).

The dose of praziquantel necessary for the treatment of adult tapeworm (*Taenia pisiformis, Echinococcus multilocularis, Echinococcus granulosus*) in dogs has been well-documented in published literature.[1,2]

A 6-consecutive monthly dosing regime was selected for effectiveness studies against *D. immitis* infections. Neither one dose nor two consecutive doses of SENTINEL SPECTRUM provided 100% effectiveness against induced heartworm (*D. immitis*) infections in dogs.

B. Substantial Evidence:

For the prevention of Heartworm Disease:

1. Laboratory Dose Confirmation Study NAH-08-0008

a. Title: Pivotal Efficacy Study of a SENTINEL SPECTRUM Chewable Formulation Administered Once or for Six Consecutive Months Following Laboratory Infection with *Dirofilaria immitis* for the Prevention of the Establishment of Adult Heartworm (*D. immitis*) Infections in Dogs

b. Investigator: John McCall, PhD
Athens, Georgia

c. Study Design: This study was conducted using principles of Good Clinical Practice (GCP).

1. Objective: Confirm the dose of SENTINEL SPECTRUM and evaluate the adult heartworm (*Dirofilaria immitis*) prevention in dogs experimentally infected with larval heartworms.

2. Study Animals: Sixteen purpose-bred Beagles between 12 and 28 weeks at start of acclimation and weighing between 6.8 and 12.2 kilograms (kgs) at initiation were included in the study.

3. Treatment Groups: One control (multi-vitamin tablet) group of 8 dogs and one SENTINEL SPECTRUM- treated group of 8 dogs (4 males and 4

[1] Thomas H, Gönnert R. 1978. The Efficacy of Praziquantel Against Cestodes in Cats, Dogs and Sheep. Res Vet Sci 24(1):20-5.

[2] Anderson FL, Crellin JR, and Cox DD. 1981. Efficacy of Praziquantel Against Immature *Echinococcus multilocularis* in Dogs and Cats. Am J Vet Res 42(11):1978-9.

females per group) were treated on Days 0, 30, 60, 90, 120, and 150.

4. Drug Administration: SENTINEL SPECTRUM was administered within 30 minutes of ingestion of food after an overnight fast to provide a milbemycin oxime dose of 0.23 – 0.34 mg/lb (0.50 – 0.74 mg/kg) based on body weights obtained 7 days prior to each dosing.

5. Measurements and Observations: On Study Day -30, all dogs were inoculated with 50 third stage *D. immitis* larvae. Adult heartworm counts were obtained at necropsy on Day 181. The general health of each dog was observed at least twice daily. Clinical observations made by a veterinarian were performed on each dog within 1 hour of test material administration, hourly for the first six hours post- treatment, and subsequently at 8, 10, and 24 hours post-treatment. Body weights were obtained pre-treatment, on Days 24, 53, 83, 112, 143, and at necropsy.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in heartworm counts in the treated group compared to the control group.

   Percent Effectiveness = $100 \times (c_c - c_t) / c_c$

   Where: c_c = Geometric mean number of worms in the control group

   c_t = Geometric mean number of worms in the treatment group

   Worm counts were logarithmically transformed and an analysis of variance (ANOVA) was performed for the treatment comparisons of interest. Treatment was included in the model as a fixed effect. Block was included in the model as a random effect.

d. Results: The geometric means and calculated effectiveness for each group are presented in Table 1 below:

Table 1. Study NAH-08-0008 Results

Treatment	Geometric Mean *D. immitis* worm count (Range)	% Effectiveness	p-value
Control (six doses)	18.8 (0 – 39)	NA	0.0002
SENTINEL SPECTRUM	0.0 (NA)	100.0	0.0002

NA = not applicable.

Statistically significant differences in the log (count+1) existed between the treated group and the control group in favor of the treated group. A minimum of five adult worms was considered to be an adequate infection and a minimum of six adequately infected control dogs was required for the study to be considered valid.

e. Adverse Reactions: One dog experienced soft feces after treatment with SENTINEL SPECTRUM on Day 60; this dog recovered uneventfully without treatment.

f. Conclusions: SENTINEL SPECTRUM is 100% effective against experimental infection with *Dirofilaria immitis* when administered once per month for 6 consecutive months after laboratory infection.

For the Prevention and Control of Flea Populations:

1. Laboratory Dose Confirmation Study NAH-03-0025

a. Title: A Pivotal Study to Evaluate the Effectiveness of 3-way Chewable Tablets (Praziquantel, Lufenuron, Milbemycin oxime) on Reproduction in the Cat Flea, *C. felis*, on Dogs

b. Investigator: Mark Holbert
Sugar Land, Texas

c. Study Design: This study was conducted using principles of Good Laboratory Practice (GLP).

1. Objective: Confirm the effectiveness of 10 mg/kg lufenuron in SENTINEL SPECTRUM in providing at least 30 days of control of reproduction in the cat flea, *C. felis*, when administered to dogs after experimental infection.

2. Study Animals: Thirty-two (16 males and 16 females) adult dogs weighing between 6.7 and 13.2 kg at study initiation were included in the study.

3. Treatment Groups:

Table 2. Study NAH-03-0025 Treatment Groups (8 dogs per group)

Treatment	Milbemycin oxime Dose	Lufenuron Dose	Praziquantel Dose
SENTINEL SPECTRUM	0.5 mg/kg	10 mg/kg	5 mg/kg
Praziquantel tablets	0 mg/kg	0 mg/kg	5 mg/kg
Control (untreated)	0 mg/kg	0 mg/kg	0 mg/kg
Lufenuron tablets	0 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: Treatments were administered once on Day 0 as outlined in Table 2 above, within 30 minutes following ingestion of a full meal after an overnight fast.

5. Measurements and Observations**:** On Days -14, 4, 11, and 18, each dog was infested with 150 newly emerged, unfed cat fleas (*C. felis*). Due to a shortage of fleas, on Day 25 each dog was infested with 100 newly emerged, unfed cat fleas. Flea eggs were collected on Days 7, 14, 21 and 28 and incubated for adult flea emergence. In addition, on Days -10, 8, 15, 22 and 29, dogs were combed to obtain flea counts. General health observations were conducted at least once daily for all dogs. On Day 0, post-dosing clinical observations were conducted hourly for the first six hours post-dose, and at hours 8, 10, 12, 18 and 24 for evidence of vomiting or other adverse signs. On Days 8, 15, 22 and 29, post-dose clinical observations were conducted hourly for 6 hours and at hours 8 and 24.

6. Statistical Methods: Insect development inhibitor effectiveness was determined on the basis of cumulative percent developmental success (CDS) in treated groups compared to the control group.

   Insect Development Inhibitor (IDI)
   CDS = cumulative number of emerged adults/cumulative number of collected eggs x 100

   Cumulative Percent Effectiveness = $100 \times (c_c - c_t)/c_c$

   Where: c_c = geometric mean of the CDS in the control group
   c_t = geometric mean of the CDS in the treatment group

   An analysis of variance (ANOVA) using log CDS was performed between the SENTINEL SPECTRUM group and the control group, and between the lufenuron group and the control group. Treatment, sex and treatment by sex were included in the model as fixed effects. Block within sex was included in the model as a random effect.

d. Results: Sex was not a significant factor in the ANOVA and the results were pooled over both sexes. Tables 3 and 4 below outline the results of the study:

Table 3. Study NAH-03-0025 Results

Treatment	Geometric Mean Emergent Flea CDS	Percent Effectiveness
SENTINEL SPECTRUM	0.2	99.5
Praziquantel tablets	52.7	0
Control	49.1	NA
Lufenuron tablets	1.0	98.0

Table 4. Study NAH-03-0025 Results

Treatment	vs. Treatment Group	p-value
SENTINEL SPECTRUM	Control	<0.0001
SENTINEL SPECTRUM	Lufenuron	0.2200
Lufenuron tablets	Control	0.0001

e. Adverse Reactions: In the SENTINEL SPECTRUM group, one dog had soft feces on Day 0, six hours post-dosing, which was likely treatment-related.

f. Conclusions: The results of this study demonstrate that SENTINEL SPECTRUM prevents flea eggs from hatching. The lufenuron in SENTINEL SPECTRUM is as effective as lufenuron alone in the prevention of flea egg hatch. Praziquantel did not interfere with the activity of lufenuron in the inhibition of flea egg development.

For the Prevention and Control of Gastrointestinal Nematodes and Cestodes:

1. Laboratory Dose Confirmation Study NAH-02-0042

a. Study Title: Pivotal Study to Evaluate SENTINEL PLUS[3] (milbemycin oxime, lufenuron, praziquantel) Chewable Tablets for the Removal of Naturally Acquired *Ancylostoma caninum* Infections in Dogs

b. Investigator: David R. Young, DVM, PhD
Turlock, California

c. Study Design: This study was conducted using principles of Good Laboratory Practice (GLP).

1. Objective: Confirm the established minimum recommended dose of 0.5 mg/kg milbemycin oxime and evaluate the effectiveness of a milbemycin oxime, lufenuron, and praziquantel combination against naturally-acquired adult hookworm (*A. caninum)* infections in dogs when administered monthly.

2. Study Animals: Twenty-four dogs between 5.6 and 72.7 months of age, weighing between 8.8 and 36.7 kg (19.4 and 80.8 lbs), and harboring naturally-acquired *A. caninum* infections, were

included in the study.

[3]SENTINEL PLUS contains the same active ingredients in the same concentrations as SENTINEL SPECTRUM. The proprietary name was changed during product development.

3. Treatment Groups:

Table 5. Study NAH-02-0042 Treatment Groups (8 dogs per group)

Treatment	Milbemycin oxime Dose	Lufenuron Dose	Praziquantel Dose
SENTINEL SPECTRUM	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (untreated)	0 mg/kg	0 mg/kg	0 mg/kg
Praziquantel tablets	0 mg/kg	0 mg/kg	5 mg/kg

4. Drug Administration: Treatments were administered once on Day 0 to their respective groups, within 30 minutes of ingestion of a meal after an overnight fast, according to the label dose of the various products, based on body weights obtained on Day -1.

5. Measurements and Observations**:** During acclimation, the presence of *A. caninum* eggs in the dogs was documented. On Day 7, all dogs were euthanized and necropsied for *A. caninum* recovery and enumeration. On Day 0, all dogs were observed hourly for six hours post-dosing, then at 8, 10, 12, 18 and 24 hours for evidence of vomiting or other adverse events. Daily observation of general health status continued throughout the study.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in hookworm counts in the treatment groups compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t)/c_c$

Where: c_c = geometric mean number of parasites in the control group
c_t = geometric mean number of parasites in the treatment group

Worm counts were logarithmically transformed and an analysis of variance (ANOVA) was performed for the treatment comparisons of interest. Treatment, sex, and treatment by sex were included in the model as fixed effects. Block within sex was included in the model as a random effect.

d. Results:
Six blocks were used for the percent effectiveness calculation and statistical analysis. Sex was not a significant factor in the ANOVA and the results were pooled over both sexes. Tables 6 and 7 below summarize the study results:

Table 6. Study NAH-02-0042 Results

Treatment	Geometric Mean	Percent Effectiveness
SENTINEL SPECTRUM	1.8	98.2
Control	100.6	NA
Praziquantel tablets	86.3	14.3

Table 7. Study NAH-02-0042 Results

Treatment	vs. Treatment Group	p-value
SENTINEL SPECTRUM	Control	0.0018
SENTINEL SPECTRUM	Praziquantel	0.0036

e. Adverse Reactions: There were no adverse reactions during this study.

f. Conclusions: SENTINEL SPECTRUM is effective against naturally acquired *A. caninum* in dogs.

Note: Studies to confirm the dose necessary for the prevention of roundworm (Toxocara canis, Toxascaris leonina) and whipworm (Trichuris vulpis) infections in dogs were conducted under the development plans for INTERCEPTOR Flavor Tabs (milbemycin oxime), NADA 140-915 (Novartis Animal Health US, Inc.), and SENTINEL Flavor Tabs (milbemycin oxime/lufenuron), NADA 141-084, (Novartis Animal Health US, Inc.). Therefore, only one study using the dose-limiting parasite (A. caninum) was necessary to demonstrate effectiveness against the gastrointestinal nematodes.

2. Laboratory Dose Confirmation Study NAH-02-0039

a. Title: Efficacy Evaluation of a 3-Way (Milbemycin oxime, Lufenuron, Praziquantel) and a 2-Way (Milbemycin oxime, Praziquantel) Chewable for the Removal of Experimentally Induced Adult *Echinococcus multilocularis* Infections in Dogs

b. Investigator: Zac Lloyd, BS
Mattawan, Michigan

c. Study Design: This study was conducted using principles of Good Laboratory Practice (GLP).

1. Objective: Confirm the dose of SENTINEL SPECTRUM and evaluate the effectiveness in dogs experimentally infected with adult *Echinococcus multilocularis*.

2. Study Animals: Thirty (15 male and 15 female) Beagle dogs varying in age from 6 to 7 months and weighing 6.6 to 8.6 kg at randomization were included in the study.

3. Treatment Groups:

Table 8. Study NAH-02-0039 Treatment Groups (10 dogs per group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
SENTINEL SPECTRUM	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (untreated)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime/lufenuron tablets	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: Test articles were administered, according to Table 8 above, once on Day 20 within 30 minutes following ingestion of a meal after an overnight fast. Dose bands were equivalent to approved milbemycin oxime/lufenuron tablet dose bands. All dogs received the appropriate sized tablet intended for dogs 5.0 – 11.4 kg.

5. Measurements and Observations: On Day 0, all dogs were orally infected with 103,360 *E. multilocularis* protoscolices. On Day 25, all dogs were euthanized and necropsied for *E. multilocularis* recovery and enumeration. All dogs were observed at least twice daily for general observations. A detailed clinical examination was performed once pre-treatment, weekly during the study, and approximately every hour for six hours, and at 8, 10, 12, and 24 hours post-dosing. Complete physical examinations were conducted once prior to *E. multilocularis* administration, prior to administration of test or control articles, and prior to euthanasia.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated groups compared to the control group.

   Percent Effectiveness = $100 \times (c_c - c_t)/c_c$

   Where: c_c = geometric mean number of parasites in the control group
   c_t = geometric mean number of parasites in the treatment group
   Worm counts were logarithmically transformed and an analysis of variance (ANOVA) was performed for the treatment comparisons of interest. The model included the fixed effects of treatment, sex, and treatment by sex interaction, along with the random effect of block within sex. If the treatment by sex interaction was significant ($p<0.05$), the percent effectiveness and analysis of variance was performed separately for each gender.

d. Results:

Sex was not a significant factor in the ANOVA and the results were pooled over both sexes. Tables 9 and 10 below summarize the study results:

Table 9. Study NAH-02-0039 Results

Treatment	Geometric Mean	Percent Effectiveness
SENTINEL SPECTRUM	0	100.0
Control	46823.1	NA
Milbemycin oxime/lufenuron tablets	52868.1	0.0

Table 10. Study NAH-02-0039 Results

Treatment	vs. Treatment Group	p-value
SENTINEL SPECTRUM	Control	<0.0001
SENTINEL SPECTRUM	Milbemycin oxime/lufenuron tablets	<0.0001

e. Adverse Reactions: In the SENTINEL SPECTRUM group, 8 dogs had reports of abnormal observations that included: soft, watery feces; discolored nose/muzzle; and skin warm to touch. In the milbemycin oxime/lufenuron group, 9 dogs had reports of abnormal observations that included emesis and soft, discolored feces with mucus. None of the dogs required treatment for any adverse reaction.

f. Conclusions: SENTINEL SPECTRUM is 100% effective against induced *E. multilocularis* infections in dogs.

3. Bioequivalence Study of Chewable and Tablet Formulations, NAH-09-0015

a. Title: A pivotal two-way cross-over bioequivalence trial comparing praziquantel in SENTINEL SPECTRUM chewable and tablet (non-final) formulations in dogs under fasting conditions

b. Investigator: Larry E. Travis, BS Fort Collins, Colorado

c. Study Design: This study was conducted using principles of Good Laboratory Practice (GLP).

1. Objective: The objective of the study was to determine if the praziquantel in a milbemycin oxime/lufenuron/praziquantel tablet formulation was bioequivalent to the praziquantel in the SENTINEL SPECTRUM formulation in healthy dogs under fasted conditions.

2. Study Animals: Twenty-four female Beagle dogs, 12 months of age or older at randomization and weighing 7.50 to 10.90 kg, were included in the study.

3. Treatment Groups:

Table 11. Study NAH-09-0015 Treatment Groups (12 dogs per group)

Treatment
SENTINEL SPECTRUM on Day 0 / 7-day washout / milbemycin oxime, lufenuron, praziquantel tablet on Day 7
Milbemycin oxime, lufenuron, praziquantel tablet on Day 0 / 7-day washout / SENTINEL SPECTRUM on Day 7

4. Drug Administration: Test articles were administered as outlined in Table 11 above after an overnight fast. Each dog was administered one milbemycin oxime/lufenuron/praziquantel tablet or one SENTINEL SPECTRUM chewable tablet containing 57 mg praziquantel.

5. Measurements and Observations:
   Plasma concentrations of Praziquantel
   Praziquantel was assayed in the collected dog plasma samples by a liquid chromatography/mass spectrophotometry/mass spectrophotometry (LC/MS/MS) method that was previously validated at PPD Laboratories, Middleton, WI.

   Safety
   General health observations were conducted once daily for all dogs. On Day 0 and Day 7, post-dosing clinical observations were conducted at 10, 20, and 30 minutes, then 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours post-dosing for evidence of vomiting or rejection of the tablet or chewable.

d. Results:

Table 12. Study NAH-09-0015 Results

Variable	SENTINEL SPECTRUM	Milbemycin oxime, lufenuron, praziquantel tablet	Bioavailability[§] (%)		Comparison p-value
			Ratio	90% CI	
$AUC_{(0-t)}$ (ng/mL·h)[†]	1125	827	136	126.1 – 146.9	<0.0001
C_{max} (ng/mL)[†]	608	344	177	152 – 204.9	<0.0001
T_{max} (h)^	1	1	NA	NA	0.3081

[†]Geometric Mean, [§]Ratio test over reference, ^Median, NA=not applicable

e. Adverse Reactions: Three adverse reactions occurred in this study. One dog had diarrhea on Day 0, 8 hours after administration of SENTINEL SPECTRUM. One dog vomited on Day 7, 1.5 hours after administration of SENTINEL SPECTRUM. One dog vomited on Day 7, four hours after administration of a milbemycin oxime/lufenuron/praziquantel tablet. All adverse reactions resolved with no treatment required.

f. Conclusions: The results of this study demonstrate that the praziquantel in SENTINEL SPECTRUM is more bioavailable than the praziquantel in the milbemycin oxime, lufenuron, praziquantel tablet formulation. The higher bioavailability of praziquantel in the chewable does not produce a significant safety concern. Additionally, the results of this study allow for a bridge to the results of the tapeworm studies (*E. granulosus*, *E. multilocularis*, and *T. pisiformis*) conducted with the milbemycin oxime/lufenuron/praziquantel tablet formulation (Studies NAH-01-0014 and NAH-01-0022; NAH 01-0015 and NAH-01-0021; NAH-01-0019 and NAH-01-0065, respectively).

4. Laboratory Dose Confirmation and Non-Interference Study NAH-01-0014 a. Title: Efficacy Evaluation of Flavored Combination Parasiticide Tablets for the Removal of Experimentally Induced Adult *Echinococcus granulosus* Infections in Dogs

b. Investigator: Steven Godin, PhD, DABT
Mattawan, MI

c. Study Design:

1. Objective: The objective of this study was to confirm the established minimum recommended dose of 5 mg/kg praziquantel, when administered with milbemycin oxime and lufenuron in the three-way combination tablets, to remove adult *Echinococcus granulosus* in dogs. Additionally, the study was intended to demonstrate that milbemycin oxime does not interfere with the effectiveness of praziquantel against *E. granulosus.*
2. Study Animals: A total of 30 purpose-bred Beagle dogs (15 males and 15 females), between 8.5 to 14 months old, weighing between 16.6 pounds (7.6 kg) and 28.4 pounds (12.9 kg) at the time of treatment, were used in this study.
3. Treatment Groups:

Table 13. Study NAH-01-0014 Treatment Groups (10 dogs per group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Milbemycin oxime/lufenuron/praziquantel tablets	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (inert ingredients)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime/lufenuron tablets	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: All dogs were dosed once, within 30 minutes of a full meal, on Day 30, according to Table 13 above.
5. Measurements and Observations**:** All dogs were determined to be healthy and heartworm negative prior to study initiation. Dogs were artificially infected (orally in canned food) with between

20,000 and 100,000 *E. granulosus* protoscolices on Day 0. All dogs were necropsied on Day 35 and the intestines examined for the presence of adult *E. granulosus* worms.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t) / c_c$

Where: c_c = Geometric mean number of worms in the control group

c_t = Geometric mean number of worms in the treatment group

Worm counts were logarithmically transformed and a mixed model analysis of variance was performed for the treatment comparison of interest.

d. Results:

Table 14. Study NAH-01-0014 Results

Treatment	Geometric Mean (range)	Percent Effectiveness
Milbemycin oxime/lufenuron/praziquantel Tablets	0[a]	100%
Control	8,130 (1,900 – 13,200)	NA
Milbemycin oxime/lufenuron Tablets	7,193 (2,600 – 14,300)	11.5%

[a]Significantly less than control and milbemycin oxime/lufenuron group geometric means ($p < .0001$)

e. Adverse Reactions: One dog vomited within 24 hours of the milbemycin oxime/lufenuron/praziquantel tablet administration. One dog experienced salivation one hour after milbemycin oxime/lufenuron/praziquantel tablets administration.

f. Conclusions: A single dose of milbemycin oxime/lufenuron/praziquantel tablets is 100% effective against adult *E. granulosus* worms in dogs. The addition of milbemycin oxime does not interfere with the effectiveness of praziquantel against *E. granulosus*. Additionally, based on the results of this study and those of the pharmacokinetics bridging study (NAH-09-0015), the SENTINEL SPECTRUM chewable tablet formulation is determined to be effective against *E. granulosus*.

5. Laboratory Dose Confirmation and Non-Interference Study NAH-01-0022

a. Title: Efficacy Evaluation of Flavored Combination Parasiticide Tablets for the Removal of Experimentally Induced Adult *Echinococcus granulosus* Infections in Dogs.

b. Investigator: Steven Godin, PhD, DABT
Mattawan, MI

c. Study Design:

1. Objective: The objective of this study was to confirm the established minimum recommended dose of 5 mg/kg praziquantel, when administered with milbemycin oxime and lufenuron in the three-way combination tablets, to remove adult *Echinococcus granulosus* in dogs.

2. Study Animals: Thirty purpose-bred, 6-month old Beagle dogs (15 males and 15 females), weighing between 14.0 pounds (6.4 kg) and 21.5 pounds (9.8 kg) at the time of treatment, were used in this study.

3. Treatment Groups:

Table 15. Study NAH-01-0022 Treatment Groups (10 dogs per group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Milbemycin oxime/lufenuron/praziquantel tablets	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (inert ingredients)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime/lufenuron tablets	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: All dogs were dosed once, within 30 minutes of a full meal, on Day 30, according to Table 15.

5. Measurements and Observations: All dogs were determined to be healthy and heartworm negative prior to study initiation. Dogs were artificially infected (orally in canned food) with between 20,000 and 100,000 *E. granulosus* protoscolices on Day 0. All dogs were necropsied on Day 35 and the intestines examined for the presence of adult *E. granulosus* worms.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t) / c_c$

Where: c_c = Geometric mean number of worms in the control group

c_t = Geometric mean number of worms in the treatment group

Worm counts were logarithmically transformed and a mixed model analysis of variance was performed for the treatment comparison of interest.

d. Results:

Table 16. Study NAH-01-0022 Results

Test Article	Geometric Mean (range)	Percent Effectiveness
Milbemycin oxime/lufenuron/praziquantel tablets	0[a]	100.0%
Control	1,091 (300 – 8,300)	NA
Milbemycin oxime/lufenuron tablets	1,429 (500 – 7,700)	0%

[a]Significantly less than control and milbemycin oxime/lufenuron group geometric means ($p < .0001$)

e. Adverse Reactions: Three dogs experienced salivation within 24 hours of milbemycin oxime/lufenuron/praziquantel tablet administration.

f. Conclusions: A single dose of milbemycin oxime/lufenuron/praziquantel tablets is 100% effective against adult *E. granulosus* worms in dogs. The addition of milbemycin oxime does not interfere with the effectiveness of praziquantel against *E. granulosus.* Additionally, based on the results of this study and those of the pharmacokinetics bridging study (NAH-09-0015), the SENTINEL SPECTRUM chewable tablet formulation is determined to be effective against *E. granulosus*.

6. Laboratory Dose Confirmation Study NAH-01-0019

a. Title: Efficacy Evaluation of Flavored Combination Parasiticide Tablets For The Removal of Natural Adult *Taenia pisiformis* (Tapeworm) Infections in Dogs

b. Clinical Investigator: Dwight Bowman, PhD
Stanwood, MI

c. Study Design:

1. Objective: To confirm the established minimum recommended dose of 5 mg/kg praziquantel, when administered with milbemycin oxime and lufenuron in the three-way combination tablets, to remove adult *Taenia pisiformis* in dogs.

2. Study Animals: Twenty-four, adult random source dogs (12 males and 12 females) weighing between 24.0 pounds (10.9 kg) and 94.5 pounds (42.9 kg) at the time of treatment were used in this study.

3. Treatment Groups:

Table 17. Study NAH-01-0019 Treatment Groups (8 dogs per group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Milbemycin oxime/lufenuron/praziquantel tablets	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (inert ingredients)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime/lufenuron tablets	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: Dogs were dosed once on day 0 within 30 minutes of a full meal, according to Table 17 above.

5. Measurements and Observations: All dogs were determined to be healthy with the exception of *T. pisiformis* infections, confirmed by fecal examination, prior to initiation of the study. All dogs were determined to be heartworm negative by antigen test prior to administration of test article. Dogs were sacrificed on Day 12 and the intestines inspected for the presence of adult *T. pisiformis* worms.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_c - c_t) / c_c$

Where: c_c = Geometric mean number of worms in the control group

c_t = Geometric mean number of worms in the treatment group

Worm counts were logarithmically transformed and a mixed model analysis of variance was performed for the treatment comparison of interest.

d. Results:

Table 18. Study NAH-01-0019 Results

Treatment	Geometric Mean	Percent Effectiveness
Milbemycin oxime/lufenuron/praziquantel tablets	0.0[a]	100.0
Control	9.5	--
Milbemycin oxime/lufenuron tablets	2.5[b]	73.7

[a] Significantly less than control and milbemycin oxime/lufenuron tablet and milbemycin oxime/lufenuron/praziquantel tablet geometric means ($p < 0.001$).

[b] Significantly less than control ($p < .0018$).

e. Adverse Reactions: There were no adverse reactions.

f. Conclusions: A single dose of milbemycin oxime/lufenuron/praziquantel

tablets is 100% effective for the removal of adult *T. pisiformis* infections in dogs. The addition of milbemycin oxime and lufenuron to praziquantel does not interfere with the effectiveness of praziquantel to remove *T. pisiformis.* Additionally, based on the results of this study and those of the pharmacokinetics bridging study (NAH-09-0015), the SENTINEL SPECTRUM chewable tablet formulation is determined to be effective against *T. pisiformis*.

7. Laboratory Dose Confirmation and Non-Interference Study NAH-01-0065 a.
Title: Efficacy Evaluation of Flavored Combination Parasiticide Tablets For The Removal of Natural Adult *Taenia pisiformis* (Tapeworm) Infections in Dogs

b. Investigator: David Young, DVM, PhD
Turlock, CA

c. Study Design:

1. Objective: To confirm the established minimum recommended dose of 5 mg/kg praziquantel, when administered with milbemycin oxime and lufenuron in the three-way combination tablets, to remove adult *Taenia pisiformis* in dogs.

2. Study Animals: Twenty-four random source, adult dogs (12 males and 12 females) weighing between 11.6 pounds (5.3 kg) and 61.9 pounds (28.1 kg) at the time of treatment were used in this study.

3. Treatment Groups:

Table 19. Study NAH-01-0065 Treatment Groups (8 dogs per group)

Treatment Article	Milbemycin dose	Lufenuron dose	Praziquantel dose
Milbemycin oxime/lufenuron/praziquantel tablets	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (inert ingredients)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime and lufenuron tablets	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: Dogs were dosed once on Day 0 within 30 minutes of a full meal, according to Table 19 above.

5. Measurements and Observations: All dogs were determined to be healthy with the exception of *T. pisiformis* infections, confirmed by fecal examination, prior to initiation of the study.

All dogs were determined to be heartworm negative by antigen test prior to administration of test article. Dogs were sacrificed on Day 12 and the intestines inspected for the presence of adult *T. pisiformis* worms.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_C - c_t) / c_C$

Where: c_C = Geometric mean number of worms in the control group

c_t = Geometric mean number of worms in the treatment group

Worm counts were logarithmically transformed and a mixed model analysis of variance was performed for the treatment comparison of interest.

d. Results:

Table 20. Study NAH-01-0065 Results

Treatment	Geometric Mean	Percent Effectiveness
Milbemycin oxime/lufenuron/praziquantel tablets	0.00[a]	100.0
Control	11.2	--
Milbemycin oxime and lufenuron tablets	7.0	37.5

[a] Significantly less than control and milbemycin oxime and lufenuron tablet geometric means ($p < .001$).

e. Adverse Reactions: There were no adverse reactions.

f. Conclusions: A single dose of milbemycin oxime/lufenuron/praziquantel tablets is 100% effective against adult *T. pisiformis* infections in dogs. The addition of milbemycin oxime to praziquantel does not interfere with the effectiveness of praziquantel against *T. pisiformis.* Additionally, based on the results of this study and those of the pharmacokinetics bridging study (NAH-09-0015), the SENTINEL SPECTRUM chewable tablet formulation is determined to be effective against *T. pisiformis*.

8. Laboratory Dose Confirmation and Non-Interference study NAH-01-0015

a. Title: Efficacy Evaluation of Flavored Combination Parasiticide Tablets for the Removal of Experimentally Induced Adult *Echinococcus multilocularis* Infections in Dogs

b. Investigator: Steven Godin, PhD, DABT
Mattawan, MI

c. Study Design:

1. Objective: To confirm the established minimum recommended dose of 5 mg/kg praziquantel, when administered with milbemycin oxime and lufenuron in the three-way combination tablets, to remove adult *Echinococcus multilocularis* in dogs.

2. Study Animals: Thirty purpose-bred Beagle dogs (15 males and 15 females), between 1 and 3 years old, weighing between 14.9 pounds (6.8 kg) and 23.4 pounds (10.6 kg) at the time of treatment were used in this study.

3. Treatment Groups:

Table 21. Study NAH-01-0015 Treatment Groups (10 dogs per group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Milbemycin oxime/lufenuron/praziquantel tablets	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (inert ingredients)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime/lufenuron tablets	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: All dogs were dosed once, within 30 minutes of a full meal, on Day 20, according to Table 21 above.

5. Measurements and Observations: All dogs were determined to be healthy and heartworm negative prior to study initiation. Dogs were artificially infected via oral gavage with between 20,000 and 100,000 *E. multilocularis* protoscolices on Day 0. All dogs were necropsied on Day 25 and the intestines examined for the presence of adult *E. multilocularis* worms.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated group compared to the control group.

Percent Effectiveness = $100 \times (c_C - c_t) / c_C$

Where: c_C = Geometric mean number of worms in the control group

c_t = Geometric mean number of worms in the treatment group

Worm counts were logarithmically transformed and a mixed model analysis of variance was performed for the treatment comparison of interest.

d. Results:

Table 22. Study NAH-01-0015 Results

Test Article	Geometric Mean (range)	Percent Effectiveness
Milbemycin oxime/lufenuron/praziquantel tablets	0[a]	100%
Control	26,349.6 (12,500 – 54,300)	NA
Milbemycin oxime/lufenuron tablets	25,981.6 (16,300 – 42, 700)	1.4%

[a]Significantly less than Control and Milbemycin oxime/lufenuron tablet geometric means ($p < .0001$)

e. Adverse Reactions: One dog had an abnormal stance (leaning) after treatment with milbemycin oxime/lufenuron/praziquantel tablets.

f. Conclusions: A single dose of milbemycin oxime/lufenuron/praziquantel tablets is 100% effective at removing adult *E. multilocularis* worms in dogs. The addition of milbemycin oxime and lufenuron does not interfere with the effectiveness of praziquantel against *E. multilocularis*. Additionally, based on the results of this study and those of the pharmacokinetics bridging study (NAH-09-0015), the SENTINEL SPECTRUM chewable tablet formulation is determined to be effective against *E. multilocularis*.

9. Laboratory Dose Confirmation and Non-Interference Study NAH-01-0021

a. Title: Efficacy Evaluation of Flavored Combination Parasiticide Tablets for the Removal of Experimentally Induced Adult *Echinococcus multilocularis* Infections in Dogs.

b. Investigator: Steven Godin, PhD, DABT
Mattawan, MI

c. Study Design:

1. Objective: To confirm the established minimum recommended dose of 5 mg/kg praziquantel, when administered with milbemycin oxime and lufenuron in the three-way combination tablets, to remove adult *Echinococcus multilocularis* in dogs.

2. Study Animals: Thirty purpose-bred Beagle dogs (15 males and 15 females), between 1 and 3 years old, weighing between 17.0 pounds (7.7 kg) and 23.4 pounds (10.6 kg) at the time of treatment were used in this study.

3. Treatment Groups:

Table 23. Study NAH-01-0021 Treatment Groups (10 dogs per group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Milbemycin oxime/lufenuron/praziquantel tablets	0.5 mg/kg	10 mg/kg	5 mg/kg
Control (inert ingredients)	0 mg/kg	0 mg/kg	0 mg/kg
Milbemycin oxime/lufenuron tablet	0.5 mg/kg	10 mg/kg	0 mg/kg

4. Drug Administration: All dogs were dosed once, within 30 minutes of a full meal, on Day 20, according to Table 23 above.

5. Measurements and Observations: All dogs were determined to be healthy and heartworm negative prior to study initiation. Dogs were artificially infected (orally in canned food) with between 20,000 and 100,000 *E. multilocularis* protoscolices on Day 0. All dogs were necropsied on Day 25 and the intestines examined for the presence of adult *E. multilocularis* worms.

6. Statistical Methods: Effectiveness was determined on the basis of the percent reduction in tapeworm counts in the treated group compared to the control group.

   Percent Effectiveness = $100 \times (c_c - c_t) / c_c$

   Where: c_c = Geometric mean number of worms in the control group

   c_t = Geometric mean number of worms in the treatment group

   Worm counts were logarithmically transformed and a mixed model analysis of variance was performed for the treatment comparison of interest.

d. Results:

Table 24. Study NAH-01-0021 Results

Treatment	Geometric Mean (range)	Percent Effectiveness
Milbemycin oxime/lufenuron/praziquantel tablets	0[a]	100%
Control	26,349.6 (10,900 – 72,600)	NA
Milbemycin oxime/lufenuron tablets	33,795.7 (13,000 – 81, 900)	0%

[a]Significantly less than control and milbemycin oxime/lufenuron tablet group geometric means ($p < .0001$)

e. Adverse Reactions: There were no adverse reactions.

f. Conclusions: A single dose of milbemycin oxime/lufenuron/praziquantel tablets is 100% effective against adult *E. multilocularis* worms in dogs. The addition of milbemycin oxime does not interfere with the effectiveness of praziquantel against *E. multilocularis*. Additionally, based on the results of this study and those of the pharmacokinetics bridging study (NAH-09-0015), the SENTINEL SPECTRUM chewable tablet formulation is determined to be effective against *E. multilocularis*.

II. Field Study NAH-02-0054

a. Title: Palatability/Acceptability Trial of 3-Way and 2-Way Chewable Formulation in Dogs

b. Investigators:

Harvey Goho, DVM
Greensboro, NC

Richard Hawkins, DVM
Durham, NC

Ronald Komich DVM
Greensboro, NC

Janet Raczkowski, DVM
Greensboro, NC

c. Study Design: This study was conducted using principles of Good Clinical Practice (GCP).

1. Objective: To determine the acceptability and safety of SENTINEL SPECTRUM administered to client-owned dogs under conditions of use.

2. Study Animals: One hundred-twenty client-owned dogs were enrolled. Three dogs were eliminated. The remaining 117 dogs consisted of 53 males and 64 females of various breeds, with ages ranging from less than 1 year to greater than 10 years, and weights ranging from 2 lbs to greater than 100 lbs. All dogs were healthy and free from conditions that could affect their appetite or feeding behavior.

3. Treatment Groups: Dogs received the appropriately-sized test article tablets based on their body weight at the time of study enrollment. In order to mask the owners and clinical investigators to the test article given, dogs received either SENTINEL SPECTRUM or a control (multi-vitamin) on treatment days.

4. Drug Administration: The test article was offered by hand for approximately 60 seconds. If it was taken from the hand and eaten, the owner recorded that result. If the test article was not taken from the hand, the owner was instructed to place it in the dog's bowl for 60 seconds. If the test article was eaten from the bowl, the owner recorded that result. If it was not eaten from the bowl, the owner was instructed to place it in the dog's mouth for 60 seconds. If the test article was not eaten when placed in the dog's mouth, the owner recorded that result and discarded the tablet.

d. Results:
The results of the study are outlined in Table 25 below:

Table 25. Study NAH-02-0054 Results
Treatment Acceptance (number of dogs) by Presentation

	Hand	Bowl	Mouth	Refuse
SENTINEL SPECTRUM	113	2	1	1

e. Adverse Reactions: There were five treatment-related adverse reactions reported in this study. One dog had facial swelling one day after receiving SENTINEL SPECTRUM. The dog was treated with an antihistamine and recovered uneventfully, but was withdrawn from the study. Two dogs vomited within one hour of administration, and another dog was anorexic one day after receiving SENTINEL SPECTRUM. One dog exhibited gagging and coughing one hour after accepting the chewable from the owner's hand.

f. Conclusions: SENTINEL SPECTRUM, administered orally under field conditions in the United States, is well-accepted in client-owned dogs.

III. TARGET ANIMAL SAFETY:

1. Acute Oral Safety Study

Note: Studies demonstrating the acute tolerability of high (10X) doses of milbemycin oxime were conducted under the development plans for INTERCEPTOR Flavor Tabs (milbemycin oxime), NADA 140-915 (Novartis Animal Health US, Inc.), and SENTINEL Flavor Tabs (milbemycin oxime/lufenuron), NADA 141-084, (Novartis Animal Health US, Inc.).

2. Laboratory Repeat Dose Target Animal Safety Study NAH-02-0066 a.

Title: A 90-day oral safety study of a milbemycin oxime, lufenuron and praziquantel combination chewable formulation with and without concomitant administration of nitenpyram (CAPSTAR) in ten week old beagle puppies.

b. Investigator: Edwin I. Goldenthal, PhD., ATS
Mattawan, MI

c. Study Design: This study was conducted using principles of Good Laboratory Practice (GLP).

1. Objective: The objective of this study was to evaluate the safety of repeat doses of SENTINEL SPECTRUM when administered to Beagle puppies at ten weeks of age at 1, 3, and 5X the maximum label exposure.

2. Study Animals: Forty purpose-bred beagles (20 males and 20 females), approximately ten weeks of age at the time of treatment, were used in this study.

3. Treatment Groups:

Table 26. Target Animal Safety Study NAH-02-0066 Treatment Groups (10 dogs per treatment group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Control (sham-dosed)	0 mg/kg	0 mg/kg	0 mg/kg
SENTINEL SPECTRUM (1X)	2.5 mg/kg	50.7 mg/kg	25.1 mg/kg
SENTINEL SPECTRUM (3X)	7.5 mg/kg	152.1 mg/kg	75.3 mg/kg
SENTINEL SPECTRUM (5X)	12.5 mg/kg	253.5 mg/kg	125.5 mg/kg

4. Drug Administration: Dogs were dosed with SENTINEL SPECTRUM every two weeks for seven treatments. Dosing occurred within 30 minutes of food consumption.

5. Measurements and Observations: Physical and neurologic examinations were conducted once prior to treatment and monthly during the course of the study. Venous blood samples for hematology and clinical chemistry, and urine samples for analysis, were collected once pre-treatment and at 1, 2 and 3 months. Ophthalmologic and electrocardiographic examinations were performed pre-test and prior to necropsy. Dogs were observed for clinical signs multiple times on the day of treatment, and then once daily at approximately 24 hour intervals for the other days of the study. Body weights were recorded at least once prior to treatment and at least weekly throughout the study, as well as immediately prior to necropsy. Food and water consumption were recorded daily throughout the study. Dogs were euthanized and necropsied at study termination. All dogs were evaluated for gross pathology and tissues from all dogs were examined histopathologically.

6. Statistical Methods: For all analyses, the individual dog was treated as the experimental unit. For variables measured at more than one time point, such as body weight, a repeated measures analysis of variance was used to test the effects treatment, treatment by time, treatment by sex, and treatment by sex by time. For variables measured once, such as organ weights, an analysis of variance was used to test the effects treatment and treatment by sex. When present, pre-treatment measurements were included as covariates. Follow-up pairwise mean comparisons between the control group and the treated groups were performed, as necessary, using linear contrasts with significance level 0.10.

d. Results: All dogs survived to termination except for one male in the 5X treated group, which was euthanized in extremis on Day 29 due to intussusception of the colon.

The incidence of ataxia, lethargy, tremors, and salivation was increased in the 3X and 5X treatment groups. Vomiting was seen in all treatment groups but had a higher incidence in the 3X and 5X treatment groups. Ataxia was observed post-dose in two dogs administered the 1X dose. Lethargy was observed in a total of six 1X treated dogs post-dose. Tremors were observed post-treatment in two 1X treated dogs. At the 5X dose, shallow breathing was noted in two dogs and inability to stand in one dog following two different doses. One male in the 3X group and two females in the 5X group had a mildly prolonged APTT at Month 1 or Month 2 during the study. The values were normal the month before and after the one abnormal result.

Clinical signs were noted following each dose and followed a dose-related pattern in onset, overall incidence, and duration. The percent of dogs observed with specific post-dose clinical observations in all groups is shown on Table 27 below. All clinical signs resolved within 24 hours.

Table 27: Study NAH-02-0066 Percent of Dogs Observed with Specific Clinical Observations by Study Day

Treatment Group	Clinical Observation	Day 1	Day 15	Day 29	Day 43	Day 57	Day 71	Day 85
Group 1 (Control)	Lethargy	-	-	-	10	-	-	-
	Vomiting	-	-	-	20	-	10	-
	Ataxia	-	-	-	10	10	-	-
	Tremors	-	-	-	-	-	-	-
	Salivation	-	-	-	-	-	-	-
Group 2 (1X)	Lethargy	10	-	-	20	30	-	10
	Vomiting	10	10	20	-	-	10	-
	Ataxia	10	-	-	-	10	-	-
	Tremors	-	-	-	10	10	-	-
	Salivation	-	-	-	-	-	-	-
Group 3 (3X)	Lethargy	30	40	20	50	20	10	20
	Vomiting	-	10	40	10	10	20	10
	Ataxia	60	30	20	50	30	30	-
	Tremors	40	20	10	30	40	20	20
	Salivation	-	30	-	-	-	-	-
Group 4 (5X)	Lethargy	60	20	44*	33	56	22	33
	Vomiting	30	40	44	44	44	44	44
	Ataxia	100	80	78	78	89	56	67
	Tremors	70	10	44	56	78	67	67
	Salivation	10	30	22	11	22	11	22
	Unable to stand	10	-	-	11	-	-	-
	Shallow Breathing	20	-	-	-	-	-	-

*Note that all percent incidences are based on 10 total dogs except the 5X treatment group. In this group the Day 29 to Day 85 values are based on 9 total dogs.

e. Conclusions: SENTINEL SPECTRUM, administered at multiples of the maximum label exposure, produce clinical signs consistent with avermectin toxicity (ataxia, tremors, lethargy, vomiting and salivation) in all treatment groups that followed a dose-related pattern in onset, overall incidence, and duration.

3. Laboratory Repeat Dose Target Animal Safety Study NAH-03-0023

a. Title: An Oral Safety Study of a Milbemycin Oxime, Lufenuron and Praziquantel Combination Chewable Formulation With and Without Concomitant Administration of Nitenpyram (CAPSTAR) in Beagle Puppies Beginning at 6 Weeks of Age

b. Investigator: M. Griggs, BS
Mattawan, Michigan

c. Study Design: This study was conducted using principles of Good Laboratory Practice (GLP).

1. Objective: To evaluate the safety of repeat doses of SENTINEL SPECTRUM

when administered to Beagle puppies at six weeks of age with at least 1, 3 and 5 times the maximum label exposure.

2. Study Animals: Sixty-four purpose-bred beagles (32 males and 32 females) approximately six weeks of age at the time of treatment were used in this study.

3. Treatment Groups:

Table 28. Treatment Groups (16 dogs per treatment group)

Treatment	Milbemycin dose	Lufenuron dose	Praziquantel dose
Control (sham-dosed)	0 mg/kg	0 mg/kg	0 mg/kg
SENTINEL SPECTRUM (1X)	2.5 mg/kg	50.7 mg/kg	25.1 mg/kg
SENTINEL SPECTRUM (3X)	7.5 mg/kg	152.1 mg/kg	75.3 mg/kg
SENTINEL SPECTRUM (5X)	12.5 mg/kg	253.5 mg/kg	125.5 mg/kg

4. Drug Administration: Dogs in groups 2 - 4 were dosed on Days 1, 15, 29 and 43 with SENTINEL SPECTRUM. Dosing occurred within 30 minutes of food consumption.

5. Measurements and Observations: Dogs were observed twice daily, at least six hours apart, for morbidity, mortality, injury, availability of food and water, inappetance, vomiting, regurgitation, and stool characteristics. On Days 1, 15, 29, and 43 detailed clinical observations were conducted immediately pre and post dose, hourly for the first six hours, then at 8, 10, 12, 18, and 24 hours after treatment (+/- 15 minutes). On days in which post-dose observations were not conducted, the dogs were observed for abnormal signs twice per day, at least six hours apart. Body weight measurements were recorded at arrival and three times weekly during the course of the study. Ophthalmoscopic and electrocardiographic examinations were performed on all dogs pre-test and prior to study termination. Physical and neurological examinations were conducted pre-test, on Day 2 and prior to study termination. Blood and urine samples for clinical pathology evaluations were collected from all dogs pre-test and prior to study termination. At study termination, complete necropsy examinations were performed, organ weights were taken, and selected tissues were microscopically examined.

6. Statistical Methods: For all analyses, the litter was treated as the experimental unit. For body weight, which was measured at multiple time points, a repeated analysis of variance was used to test the effects treatment and treatment by time. For variables measured once, such as organ weights, an analysis of variance was used to test the treatment effect. When present, pre-treatment measurements were included as covariates. Follow-up pair-wise mean comparisons between the control group and the treated groups were performed, as necessary, using linear contrasts with significance level 0.10.

d. Results: All dogs survived to study completion. A dose-dependent increase in the incidence of treatment associated clinical signs (ataxia, decreased activity, salivation, vomiting and tremors) was typically observed in dogs within 24 hours of test article administration. Ataxia was the most common clinical finding in the 3X and 5X treatment groups, with a greater incidence in the 5X groups overall. Ataxia was observed on days the test article was administered from 2 to 18 hours post-dose. Decreased activity was observed between 2 and 18 hours post-dose

in all treatment groups. Salivation was observed in all treatment groups, but most frequently in the 5X group, usually within 10 hours post-dose. Tremors were observed once in a 5X SENTINEL SPECTRUM-treated female on Day 15, 4 hours following treatment. A finding of splayed hind limbs was noted one time in one dog in the 5X treatment group. Vomiting was observed in the 5X treatment group.

Table 29 below summarizes the percentage of dogs with specific clinical findings by treatment days. The values are calculated based on 16 dogs per group per treatment day.

Table 29. Study NAH-03-0023 Results Percent of Dogs with Specific Clinical Observations by Study Day

Group	Clinical Observation	Day 1	Day 15	Day 29	Day 43
Group 1 (0X) Control	Decreased activity	-	-	-	-
	Ataxia	-	-	-	-
	Salivation	-	6.25	-	-
	Emesis	-	-	-	-
Group 2 (1X)	Decreased activity	-	-	-	6.25
	Ataxia	-	-	-	-
	Salivation	-	6.25	-	-
	Emesis	-	-	-	-
Group 3 (3X)	Decreased activity	-	-	6.25	25.0
	Ataxia	-	25.0	6.25	12.5
	Salivation	-	-	-	18.75
	Emesis	-	-	-	-
Group 4 (5X)	Decreased activity	37.5	68.75	87.5	75.0
	Ataxia	37.5	87.5	87.5	87.5
	Splayed hind limbs	-	6.25	-	-
	Tremors	-	6.25	-	-
	Salivation	-	12.5	12.5	37.5
	Emesis	-	-	25.0	25.0

The majority of dogs with test article-related findings occurred in the 5X treatment group. There was a lower rate of occurrence on Day 1 compared to the other treatment days.

e. Conclusions: SENTINEL SPECTRUM, administered at multiples of the maximum label exposure, produced clinical signs consistent with avermectin toxicity (ataxia, tremors, decreased activity, vomiting and salivation) that followed a dose-related pattern in onset, overall incidence, and duration.

IV. HUMAN FOOD SAFETY:

This drug is intended for use in dogs, which are non-food animals. Because this new animal drug is not intended for use in food producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to SENTINEL SPECTRUM:

"Not for human use. Keep this and all drugs out of the reach of children."

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that SENTINEL SPECTRUM, when used according to the label, are safe and effective for the prevention of heartworm disease caused by *Dirofilaria immitis*; for the prevention and control of flea populations (*Ctenocephalides felis)*; and for the treatment and control of adult roundworm *(Toxocara canis, Toxascaris leonina)*, adult hookworm *(Ancylostoma caninum)*, adult whipworm *(Trichuris vulpis),* and adult tapeworm *(Taenia pisiformis, Echinococcus multilocularis* and *Echinococcus granulosus)* infections in dogs and puppies two pounds of body weight or greater and six weeks of age and older.

A. Marketing Status:

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). Adequate directions for use cannot be written because the product is indicated for the prevention of heartworm infections (*Dirofilaria immitis*) in dogs, which requires veterinary examination and testing to ensure dogs are negative for adult heartworm disease prior to administration of the product.

B. Exclusivity:

Under Section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.